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METHOD FOR CHARACTERIZATION OF SELECTIVITY IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

III. RETENTION BEHAVIOUR IN GRADIENT-ELUTION CHROMATOGRAPHY: APPLICATION TO THE CHROMATOGRAPHY OF PESTICIDE COMPOUNDS

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SUMMARY

The method introduced recently for the characterization and prediction of absolute and relative retention in reversed-phase liquid chromatography under isocratic conditions has been applied to gradient-elution chromatography. The method is based on two indices (one lipophilic, n_{ce}, and the other polar, q_i, to characterize the behaviour of each solute in methanol–water mobile phases of various compositions. The indices n_{ce} and q_i of phenylurea and triazine herbicides were found to be composed of additive contributions of structural elements (functional groups) in the molecules of the solutes. Using the present approach, it is possible to predict retention volumes in gradient-elution chromatography with a precision comparable to those under isocratic conditions (*i.e.*, less than 10% relative for most compounds tested).

Simultaneous adjustment of the initial mobile phase composition and the slope of the linear gradient makes it possible to control to a certain extent the selectivity in gradient-elution chromatography using binary solvent gradients and offers an alternative approach to the use of multi-solvent gradients for selectivity control. This method may be especially useful in optimizing gradient-elution separations of pairs of compounds with positive differences in both indices n_{ce} and q_i, $\Delta n_{ce} > 0$ and $\Delta q > 0$. Selection of the optimal gradient profile is illustrated on a practical example of gradient-elution chromatography of phenylurea herbicides.

INTRODUCTION

Prediction of retention in liquid chromatography by calculation is potentially useful both for the optimization of separations and for the identification of separated compounds. Theoretical models for reversed-phase liquid chromatography based on solvophobic theory[1,2] or on molecular statistical theory[3] make use of a number of physico-chemical parameters, which often are not available. Consequently, these

models are not suitable for direct predictions of retention. Some methods try to predict retention in reversed-phase systems from correlations of retention data with various structural parameters, such as with hydrophobic substituent constants, molecular connectivity indices, Hammett's constants, the shape or volume of the solute molecules or with a combination of these parameters[4–8]. These approaches are limited by the availability of the structural parameters and require a knowledge of correlation constants for each mobile phase composition. The prediction of retention based on a scale of retention indices similar to Kováts' retention indices, based on alkyl aryl ketones[9,10] or on 2-alkanones[11,12] as the calibration standards may be applied only within a limited composition range of mobile phases. This approach, like the prediction of retention based on interaction indices[13,14], cannot take full account of specific solute–solvent interactions and makes it difficult to predict selectivity changes with changing mobile phase composition.

To overcome this inconvenience, a method has been introduced recently that makes use of a suitable homologous series (such as *n*-alkylbenzenes) to calibrate the retention scale and of two indices to characterize the retention of each solute over a wide range of mobile phase composition[15]. One index, n_{ce}, accounts for the hydrophobicity of a solute and the other, q_i, for the polarity of its functional group(s) interacting with mobile phase components[16]. It has been shown in preceding parts of this series[16,17] that this method predicts correctly the selectivity changes induced by changes in the mobile phase composition and may be used to predict relative and absolute retentions under isocratic conditions in binary and ternary mobile phases with a relative error of less than 10–20%[17]. It was further found that the lipophilic and polar indices used in this approach may be calculated from the additive contributions of structural elements, *i.e.*, substituents in simple aromatic compounds, such as methylbenzenes, chlorobenzenes and chloroanilines[16].

It was the purpose of this work to investigate the possibilities of the characterization of selectivity, the prediction of retention and the optimisation of separation conditions (gradient profile) in gradient-elution reversed-phase chromatography, using the method based on the lipophilic and polar indices. To verify the validity of additive rules for the contributions to the lipophilic and polar indices, phenylurea and triazine herbicides were selected as test compounds as these have more complex structures than the compounds studied previously.

Various high-performance liquid chromatographic (HPLC) techniques have been applied previously for separations of these herbicide compounds in mixtures and for determining individual herbicides in industrial products or in environmental samples. Phenylurea herbicides were chromatographed on columns packed with unmodified silica gel[18–21], with chemically bonded alkylsilica materials[19,20,22–33], bonded nitrile[23,32] and amino[21] stationary phases or with organic gels[34]. Reversed-phase chromatography[28,35–39], chromatography on unmodified silica gel[40,41] and on medium-polarity chemically bonded phases[39,42,43] yielded successful separations of triazine herbicides.

THEORETICAL

In the recently derived[15] description of retention in reversed-phase chromato-

graphy over a wide range of mobile phase compositions, a set of appropriate homologues, such as n-alkylbenzenes, is used to calibrate the retention scale:

$$\log k' = (a_0 + a_1 n_{ce})(1 - px) - q_i x \qquad (1)$$

where k' is the capacity factor and x is the volume concentration of the organic solvent in aqueous–organic mobile phases. The retention of each compound in this scale is characterized by two indices, n_{ce} and q_i. The calibration constants a_0, a_1 and p apply for a given column and various compositions of binary mobile phases containing water and a given organic solvent, such as methanol or acetonitrile, and are determined from the retention–composition equations for calibration homologues, provided that $\log k'$ varies linearly with x[44]:

$$\log k' = a - mx \qquad (2)$$

In a homologous series, the constants a and m in eqn. 2 increase linearly with increasing number of carbon atoms, n_c, in the alkyl chains of the individual homologues:

$$a = a_0 + a_1 n_c \qquad (3a)$$

$$m = m_0 + m_1 n_c \qquad (3b)$$

$$m = q + pa \qquad (3c)$$

The indices n_{ce} and q_i of an arbitrary compound are equivalent to constants n_c and q that apply to the calibration homologous series. The lipophilic index n_{ce} characterizes the hydrophobicity of the compound and the polarity index q_i the polarity of functional group(s) in the solute interacting with mobile phase components.

If the linear $\log k'$ versus x equation (eqn. 2) is not suitable for describing retention in a given chromatographic system, a quadratic equation may be used as the basis of retention characterization[15].

It has been shown that the indices n_{ce} and q_i increase or decrease regularly with the number of chlorine atoms and methyl groups in mono- to hexa(or penta) methylbenzenes, chlorobenzenes, chloroanilines and chlorophenols[16]. This means that, at least for some compound classes, the indices n_{ce} and q_i may be calculated from additive contributions of the individual substituents i, Δn_{ci}, Δq_i:

$$n_{ce} = n_{ce(0)} + \sum_i \Delta n_{ci} \qquad (4a)$$

$$q_i = q_{i(0)} + \sum_i \Delta q_i \qquad (4b)$$

where $n_{ce(0)}$ and $q_{i(0)}$ are the values of n_{ce} and q_i for the basic structural element in a given compound class. A lipophilic and a polar contribution to the relative retention of a pair of compounds, i and j, may be distinguished from each other on the basis of the differences in the indices for compounds i and j[17]:

$$\Delta n_c = n_{ce,j} - n_{ce,i} \tag{5a}$$

$$\Delta q = q_{i,j} - q_{i,i} \tag{5b}$$

The theory developed for chromatography under isocratic conditions may be extended to gradient-elution reversed-phase chromatography in a straightforward manner. If linear concentration gradients of the organic solvent in aqueous–organic mobile phases are used, net retention volumes, V'_g, of solutes eluted before the end of the gradient may be calculated from the following equation, provided that the delay of the gradient between the mixing port and the top of the column can be neglected[45-47]:

$$V'_g = \frac{1}{mB} \cdot \log[2.31 mBV_M \cdot 10^{(a - mA)} + 1] \tag{6}$$

where V_M is the column dead volume, A is the initial concentration of the organic solvent in the mobile phase at the start of the gradient and B is the slope of the concentration gradient described by the following gradient function:

$$x = A + BV \tag{7}$$

where V is the volume of eluate from the start of the gradient elution.

Band widths, w_g, of compounds eluted using linear concentration gradients in reversed-phase chromatography may be calculated using the following equation[45,47]:

$$w_g = \frac{4V_M}{\sqrt{n}} \cdot \{1 + [2.31 mBV_M + 10^{-(a - mA)}]^{-1}\} \tag{8}$$

where n is the plate number of the column used.

After introducing eqns. 9a and b, analogous to eqns. 3a,c:

$$a = a_0 + a_1 n_{ce} \tag{9a}$$

$$m = q_i + p(a_0 + a_1 n_{ce}) \tag{9b}$$

into eqn. 6, the following relationship for net retention volumes in gradient-elution chromatography as a function of n_{ce} and q_i is obtained:

$$V'_g = \frac{1}{B[q_i + p(a_0 + a_1 n_{ce})]} \cdot \log \{2.31 BV_M[q_i + p(a_0 +$$

$$+ a_1 n_{ce})] \cdot 10^{[(a_0 + a_1 n_{ce})(1 - Ap) - \Delta q_i]} + 1\} \tag{10}$$

Eqn. 8 may be adapted in an analogous manner to describe the dependence of band widths in gradient-elution chromatography on the indices n_{ce} and q_i.

In contrast to isocratic-elution liquid chromatography, the ratio of net retention volumes in gradient-elution chromatography is not a good measure of separation

selectivity, because it depends on the profile of the gradient. Instead, the difference in retention volumes of the pair of compounds i and j, $\Delta V_g = V''_{g,j} - V''_{g,i}$, is better suited-to characterization of the separation, as the band widths of different compounds eluted in a single gradient run are approximately constant if the gradient profile is chosen appropriately[47,48]. Eqn. 10 may be easily adapted to describe the difference in retention volumes as a function of the indices n_{ce} and q_i of the solutes i and j, but it can be shown that ΔV_g depends not only on Δn_c and Δq, but also on the absolute values $n_{ce,i}$ and $q_{i,i}$. The form of the equation for ΔV_g does not allow the influence of various factors on ΔV_g to be analysed in a straightforward manner. Therefore, these effects are illustrated by model examples in Fig. 1, where calculated ΔV_g values are plotted against Δn_c for various hypothetical combinations of Δq, $q_{i,i}$ and $n_{ce,i}$ for a typical column and linear gradient beginning at zero concentration of the organic solvent in water, A. ΔV_g increases with increasing Δn_c or with decreasing Δq at constant $n_{ce,i}$ and $q_{i,i}$. The latter indices influence both the difference ΔV_g and the rate of its increase, which diminishes significantly with increasing $n_{ce,i}$ and increases slightly with increasing $q_{i,i}$. The differences ΔV_g are higher at lower $n_{ce,i}$ and at higher $q_{i,i}$, provided that Δn_c and Δq are constant, which means that greater structural differences between two more strongly retained compounds than between two compounds with lower retention are required in order to achieve an equal difference in retention volumes under identical gradient-elution conditions.

In the chromatography of such pairs of compounds for which both the Δn_c and Δq differences are positive, i.e., if one of these compounds has a bulkier hydrocarbon part and a more polar functional group (or groups) than the other compound (curves 3, 6, 7 and 8), the order of elution may change depending on the Δn_c, Δq, $n_{ce,i}$ and $q_{i,i}$ values. As Fig. 1 shows, a decrease in ΔV_g values with increasing Δn_c may be expected up to a certain value of Δn_c, where the compounds are co-eluted in a single peak. ΔV_g increases from zero when Δn_c further increases, but the order of elution is reversed. For such pairs of compounds, a reversal in the elution order may also be expected on changing the initial concentration of the organic solvent in the mobile phase, A, or on changing the slope of the gradient, B.

If the additivity rules (eqns. 4a and 4b) apply accurately enough for the set of compounds being studied, it should be possible to predict by calculation retention volumes, order of elution, differences in retention volumes and resolution in reversed-phase gradient-elution chromatography from the structural contributions.

EXPERIMENTAL

The equipment used included two Model 6000 A pumps, a Model 660 gradient controller, an U6K injector and a Model 440 UV detector operated at 254 nm (all from Waters Assoc., Milford, MA, U.S.A.), and a TZ 4221 line recorder (Laboratory Instrument Works, Prague, Czechoslovakia). A Model R 401 differential refractometer (Waters Assoc.) was used for measuring column dead volumes, V_M.

Two stainless-steel columns were packed in the laboratory with spherical octadecylsilica gel Silasorb SPH C_{18}, 7.5 μm (Lachema, Brno, Czechoslovakia) using a high-pressure slurry packing technique. Column A: 300 mm $\times$ 3.6 mm I.D., $V_M = 2.42$ cm^3 (mean value) for methanol and ^2H_2O as dead volume markers, refractometric detection, ε_T (total column porosity) = 0.79, plate number $n = 4100$
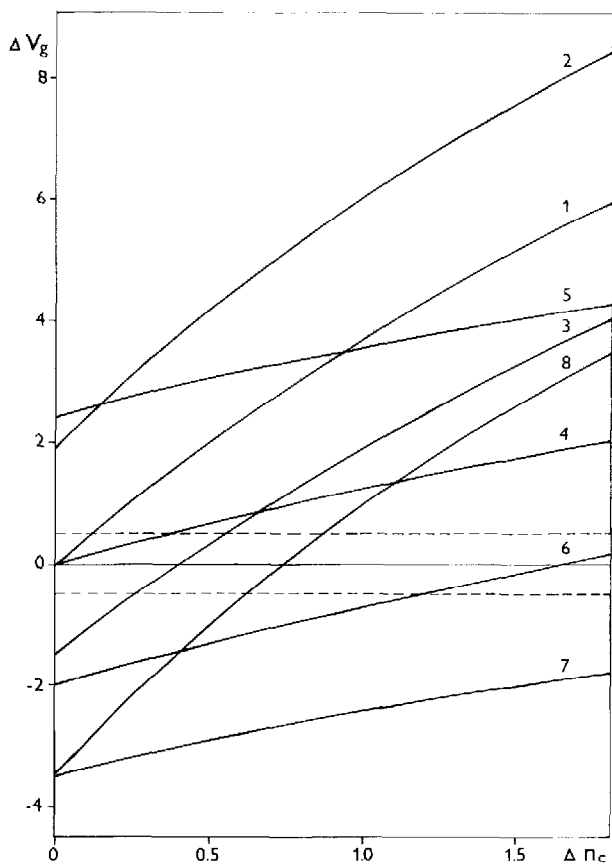
Fig. 1. Dependence of the difference in retention volumes in gradient-elution liquid chromatography, ΔV_g, on the difference in the lipophilic indices, Δn_c, of an adjacent pair of compounds for various values of the indices $n_{ce,i}$, $q_{i,i}$ and Δq_i. Model curves were constructed for a linear gradient of an organic solvent in water (gradient time = 30 min; flow-rate = 1 cm³ min⁻¹; $A = 0$; $B = 0.03333$); Dead volume of the column, $V_M = 3.18$ cm³.

	Curve							
	1	2	3	4	5	6	7	8
$n_{ce,i}$	−1	−1	−1	3	3	3	3	−1
$q_{i,i}$	1.5	1.5	1.5	1.5	1.5	1.5	0.2	0.2
Δq_i	0	−0.5	0.5	0	−0.5	0.5	0.5	0.5

at 1 cm³/min in methanol for benzene as the solute. Column B: 300 mm × 4.1 mm I.D., $V_M = 3.18$ cm³, $\varepsilon_T = 0.80$, $n = 4600$, conditions as for column A.

The mobile phases were prepared by mixing water (doubly distilled in glass with addition of potassium permanganate) with methanol (spectroscopic grade, Lachema) in the required volume ratios for isocratic experiments, where a single M 6000 A pump and column A were employed. Column B was used in isocratic and

gradient-elution experiments for comparison of experimental retention volumes with the data calculated from the constants determined using column A. In gradient-elution experiments, water was used as component a and methanol as component b, the volume ratio of which in the mobile phase was changed with time by the M 660 controller.

Table I lists the compounds used as the sample solutes. The mobile phase used in isocratic experiments was employed as the sample solvent; 15% v/v methanol in water served as the sample solvent in gradient-elution experiments. Sample volumes of 5 μl were injected. The capacity factors were calculated as $k' = V_R/V_M - 1$ from the arithmetic means of two or three experimental retention volumes, V_R, for each of the mobile phases tested.

RESULTS AND DISCUSSION

Structural dependence of retention of herbicide compounds; additive increments to the indices n_{ce} and q_i
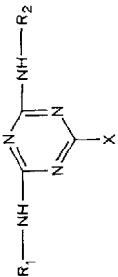
The retention volumes, V_R, of all the test solutes listed in Table I were measured on column A in mobile phases containing 40–80% methanol in water. Some of these experimental values are given in Table IV. From the experimental plots of log k' *versus* concentration of methanol in mobile phase, x, the parameters a and m in eqn. 2 were determined by linear regression analysis and are listed in Table II. As the values of the correlation coefficients show, eqn. 2 is well suited to describe the retention of the compounds studied over the mobile phase composition range tested.

The plots of the parameters a and m of calibration homologous *n*-alkylbenzenes (methylbenzene to *n*-butylbenzene) *versus* n_c were used for determining the constants a_0, a_1, m_0, m_1, q and p in eqns. 3a–c. Using these values for calibration of the retention scale, the indices n_{ce} and q_i and the relative indices with respect to toluene as the standard were calculated for each solute tested using eqns. 9a and b and 5a and b and are listed in Table II.

Retention of phenylurea herbicides and related compounds generally increases with increasing number and size of alkyl substituents and of halogen atoms (a bromine substituent contributes more than a chlorine atom to retention), but it decreases significantly if a hydroxy substituent is present on the phenyl ring. A methoxy substituent on the phenyl ring decreases the retention slightly, in contrast to a methoxy substituent on the urea nitrogen, which increases the retention more than a methyl group. This is probably caused by interactions between the neighbouring oxygen and nitrogen atoms, which decrease the solute polarity.

In the class of phenylurea herbicides, additive structural contributions to the indices n_{ce} and q_i are observed. It was possible to determine additive contributions of various substituents, Δn_{ci} and Δq_i, to these indices. These values are given in Table III, together with the values of $n_{ce(0)}$ and $q_{i(0)}$ for the unsubstituted phenylurea as the basic structural element (eqns. 4a and b). Most of the experimental n_{ce} and q_i values agree very well with those calculated from eqns. 4a and b (Table III). The additive contributions to n_{ce} for substituents on the phenyl group of substituted phenylureas agree with the contributions in simple substituted benzenes determined earlier on octadecylsilica columns[16], *e.g.*, $\Delta n_{ci} = 0.94$ for a methyl, 1.11 for a chloro and -1.29 for a phenolic hydroxy substituent. Alkyl, halogen and methoxy substituents on the

TABLE I

LIST OF CHROMATOGRAPHED COMPOUNDS

Type	No.	Compound
Calibration standards	1	Benzene
	2	Toluene
	3	Ethylbenzene
	4	n-Propylbenzene
	5	n-Butylbenzene
Phenylurea herbicides and related compounds:	6	Hydroxymetoxuron: $R_1 = CH_3$; $R_2 = CH_3$; $X_1 = Cl$; $X_2 = OH$
	7	Fenuron: $R_1 = CH_3$; $R_2 = CH_3$; $X_1 = H$; $X_2 = H$
	8	Metoxuron: $R_1 = CH_3$; $R_2 = CH_3$; $X_1 = Cl$; $X_2 = OCH_3$
	9	N'-(3-Chloro-4-methylphenyl)-N-methylurea: $R_1 = H$; $R_2 = CH_3$; $X_1 = Cl$; $X_2 = CH_3$
	10	Diuron: $R_1 = CH_3$; $R_2 = CH_3$; $X_1 = Cl$; $X_2 = Cl$
	11	Linuron: $R_1 = CH_3$; $R_2 = OCH_3$; $X_1 = Cl$; $X_2 = Cl$
	12	Chlorbromuron: $R_1 = CH_3$; $R_2 = OCH_3$; $X_1 = Cl$; $X_2 = Br$
	13	Neburon: $R_1 = CH_3$; $R_2 = C_4H_9$; $X_1 = Cl$; $X_2 = Cl$
	14	Desfenuron: $R_1 = H$; $R_2 = CH_3$; $X_1 = H$; $X_2 = H$
	15	Fluometuron: $R_1 = CH_3$; $R_2 = CH_3$; $X_1 = CF_3$; $X_2 = H$
	16	Chlortoluron: $R_1 = CH_3$; $R_2 = CH_3$; $X_1 = Cl$; $X_2 = CH_3$
	17	Monolinuron: $R_1 = CH_3$; $R_2 = OCH_3$; $X_1 = H$; $X_2 = Cl$
	18	Deschlormetoxuron: $R_1 = CH_3$; $R_2 = CH_3$; $X_1 = H$; $X_2 = OCH_3$
	19	Metobromuron: $R_1 = CH_3$; $R_2 = OCH_3$; $X_1 = H$; $X_2 = Br$
	20	Isoproturon: $R_1 = CH_3$; $R_2 = CH_3$; $X_1 = H$; $X_2 = i\text{-}C_3H_7$
Carbamate pesticides	21	Methomyl [1-methylthio-O-(N-methylcarbamoyl)acetaldoxime]
	22	Triallat (N,N-diisopropyl-S-2,3,3-trichlorallylthiocarbamate)
	23	Carbofuran (2,3-dihydro-2,2-dimethylbenzofuran-7-yl-N-methylcarbamate)
	24	Thiofanox (3,3-dimethyl-1-methylthio-2-butanone-O-methylaminocarbonyloxime)
	25	Phenmedipham [O-(3-methoxycarbonylanilino)-N-(3'-methylphenyl)carbamate]
	26	Desmedipham [3-(ethoxycarbamoylanilino)-N-phenylcarbamate]
Triazine herbicides:	28	Simazin: $X = Cl$; $R_1 = C_2H_5$; $R_2 = C_2H_5$
	29	Atrazin: $X = Cl$; $R_1 = C_2H_5$; $R_2 = i\text{-}C_3H_7$
	30	Terbutylazin: $X = Cl$; $R_1 = C_2H_5$; $R_2 = t\text{-}C_4H_9$
	31	Methoprotryn: $X = SCH_3$; $R_1 = CH_3O(CH_2)_3$; $R_2 = i\text{-}C_3H_7$
	32	Desmetryn: $X = SCH_3$; $R_1 = CH_3$; $R_2 = i\text{-}C_3H_7$
	33	Prometryn: $X = SCH_3$; $R_1 = i\text{-}C_3H_7$; $R_2 = i\text{-}C_3H_7$
	34	Terbutryn: $X = SCH_3$; $R_1 = C_2H_5$; $R_2 = t\text{-}C_4H_9$

TABLE II

EXPERIMENTAL CONSTANTS a AND m OF EQN. 2 AND THE INDICES n_{ce}, q_i, Δn_c AND Δq (RELATED TO TOLUENE, EQNS. 5a AND b) ON SILASORB SPH C$_{18}$ IN METHANOL–WATER MOBILE PHASES

The indices n_{ce} and q_i were calculated using eqns. 9a and b. r_k = correlation coefficient. Numbers of compounds as in Table I. Constants a_0, a_1 and p for alkylbenzenes as calibration standards were determined by linear regression analysis of the plots of a versus n_c, m versus n_c and m versus a (eqns. 3a–c). a_0 = 1.948; a_1 = 0.577; r_k = 0.9991. q = 0.969; p = 0.890; r_k = 0.9997. m_0 = 2.704; m_1 = 0.514; r_k = 0.9995.

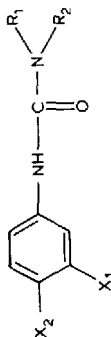| Type	Compound No.	a	m	r_k	n_{ce}	q_i	Δn_c	Δq
Calibration standards: n-alkylbenzenes	1	2.749	1.971	0.9995	0.04	1.00	−0.98	0.04
	2	3.215	2.535	0.9997	1.02	0.96	0	0
	3	3.714	3.077	0.9996	1.96	0.91	0.94	−0.05
	4	4.286	3.706	0.9993	3.05	0.99	2.03	0.03
	5	4.737	4.250	0.9984	3.99	0.96	2.97	0.00
Phenylurea herbicides and related compounds	6	1.419	3.800	0.9996	−0.92	2.54	−1.94	1.58
	7	1.287	2.786	0.9998	−1.15	1.64	−2.17	0.68
	8	1.947	3.570	0.9996	0.00	1.84	−1.02	0.88
	9	2.518	3.776	0.9997	0.99	1.53	−0.03	0.57
	10	2.811	3.995	0.9998	1.50	1.49	0.48	0.53
	11	3.224	4.366	0.9993	2.21	1.50	1.19	0.54
	12	3.366	4.494	0.9995	2.46	1.50	1.44	0.54
	13	4.043	5.110	0.9990	3.63	1.51	2.61	0.55
	14	1.140	2.615	0.9990	−1.40	1.60	−2.42	0.64
	15	2.661	4.072	0.9994	1.24	1.70	0.22	0.74
	16	2.659	3.952	0.9998	1.23	1.58	0.21	0.62
	17	2.499	3.820	0.9999	0.96	1.60	−0.06	0.64
	18	1.326	3.028	0.9995	−1.08	1.85	−2.10	0.89
	19	2.600	3.833	0.9996	1.13	1.52	0.11	0.56
	20	2.830	4.116	0.9999	1.53	1.60	0.51	0.64
Carbamate pesticides	21	0.754	2.625	0.9955	−2.07	1.95	−3.09	0.99
	22	4.550	5.060	0.9997	4.51	1.01	3.49	0.99
	23	2.267	3.761	0.9996	0.55	1.74	−0.47	0.78
	24	2.493	3.771	0.9996	0.94	1.55	−0.08	0.59
	25	3.503	5.133	0.9999	2.70	2.01	1.68	1.05
	26	3.349	4.953	0.9991	2.43	1.97	1.41	1.01
Triazine herbicides	28	2.124	3.392	0.9995	0.30	1.50	−0.72	0.54
	29	2.629	3.819	0.9999	1.18	1.48	0.16	0.52
	30	3.259	4.371	0.9989	2.27	1.47	1.25	0.51
	31	3.262	4.466	0.9984	2.28	1.56	1.26	0.60
	32	2.675	3.824	0.9993	1.26	1.44	0.24	0.48
	33	3.609	4.634	0.9989	2.88	1.42	1.86	0.46
	34	3.732	4.750	0.9996	3.09	1.43	2.07	0.47

TABLE III

ADDITIVE CONTRIBUTIONS, Δn_{ci} AND Δq_i, OF SUBSTITUENTS TO n_{ce} AND q_i VALUES FOR PHENYLUREA AND TRIAZINE HERBICIDES

$n_{ce(0)}$ and $q_{i(0)}$ are the values of n_{ce} and q_i indices for unsubstituted structural basis, *i.e.*, phenylurea and 4,6-diamino-1,3,5-triazine. Numbers of compounds and substituents X, X_1, X_2, R_1 and R_2 as in Table I. Exp. = experimental values; calc. = values calculated using additive rules (eqns. 4a and b).

(A) Phenylurea herbicides				*(B) Triazine herbicides*		
$n_{ce(0)} = -1.40$; $q_{i(0)} = 1.60$				$n_{ce(0)} = -3.53$; $q_{i(0)} = 1.45$		
Contributions:	Δn_{ci}	Δq_i		Contributions:	Δn_{ci}	Δq_i
(1) For substituents R_1, R_2:				(1) For substituents X:		
CH_3	0.25	0.04		Cl	1.33	−0.08
OCH_3	0.96	0.04		SCH_3	2.15	−0.14
C_4H_9	2.38	0.06				
(2) For substituents X_1, X_2:				(2) For substituents R_1, R_2:		
CH_3	1.05	0		CH_3	0.25	0.04
isoC_3H_7	2.68	0		C_2H_5	1.25	0.06
OCH_3	−0.05	0.24		iso-C_3H_7	2.13	0.06
OH	−1.10	0.97		tert.-C_4H_9	3.22	0.06
Cl	1.33	−0.08		$CH_3O(CH_2)_3$	1.69	0.13
Br	1.50	−0.1				
CF_3	2.39	0.06				

Compound No.	n_{ce} *calc.*	n_{ce} *exp.*	q_i *calc.*	q_i *exp.*	*Compound No.*	n_{ce} *calc.*	n_{ce} *exp.*	q_i *calc.*	q_i *exp.*
6	−0.92	−0.92	2.53	2.54	28	0.30	0.30	1.49	1.50
7	−1.15	−1.15	1.64	1.64	29	1.18	1.18	1.49	1.48
8	0.13	0.00	1.80	1.84	30	2.27	2.27	1.49	1.47
9	0.98	0.99	1.52	1.53	31	2.44	2.28	1.50	1.56
10	1.51	1.50	1.48	1.49	32	1.00	1.26	1.41	1.44
11	2.22	2.21	1.48	1.50	33	2.88	2.88	1.43	1.42
12	2.39	2.46	1.46	1.50	34	3.09	3.09	1.43	1.43
13	3.64	3.63	1.50	1.51					
14	−1.40	−1.40	1.60	1.60					
15	1.24	1.24	1.70	1.70					
16	1.23	1.23	1.56	1.58					
17	0.89	0.96	1.56	1.60					
18	−1.20	−1.08	1.88	1.85					
19	1.06	1.13	1.54	1.52					
20	1.53	1.53	1.64	1.60					

phenyl have no or only a slight influence on the q_i indices of substituted phenylureas, as with simple substituted benzenes, but the phenolic group increases q_i more for substituted phenylureas than for substituted benzenes. The q_i indices of all the phenylurea herbicides studied, with the exception of hydroxymetoxuron, which possesses a phenolic group, are close to one another and to the values determined earlier for linuron and chlorobromuron on a Silasorb C_8 column[16].

The retention of triazine herbicides increases with increasing number and length of alkyl substituents. Alkoxy substituents contribute to the retention less than alkyl substituents of comparable length. A methylthio group on the triazine ring increases the retention more than a chlorine atom.

Additive contributions of the individual substituents to the n_{ce} and q_i indices of triazine herbicides are given in Table III. Here, only the values of $n_{ce(0)}$, $q_{i(0)}$, Δn_{ci} and Δq_i for methylthio and Δn_{ci} for isopropyl and *tert.*-butyl groups were determined from the experimental data. Other additive contributions were taken from previous experiments with other compounds: the contributions Δn_{ci} and Δq_i for a chloro substituent on the triazine ring were set equal to those for a chloro substituent on the phenyl group of phenylurea herbicides and Δn_{ci} and Δq_i for methyl substituents on amine nitrogen were set equal to those for a methyl group on phenylurea nitrogen; $\Delta n_{ci} = 1$ was set for the contribution of a methylene group and $\Delta n_{ci} = 1.69$ for a $CH_3O(CH_2)_3$–substituent was calculated using the values 0.62 for methyl and methylene groups, -0.32 for the ether oxygen[16] and $0.25 \cdot 0.62 = 0.15$ for the methylene group bond to the nitrogen. Δq_i for alkyl groups with more than one carbon atom were set to 0.06 (*i.e.*, the value for *n*-butyl on the nitrogen of phenylurea herbicides) and Δq_i for a $CH_3O(CH_2)_3$–substituent was increased to 0.13 for the contribution of the ether oxygen, which is 0.07[16].

An increase in retention with increasing number and size of alkyl and halogen substituents in carbamate pesticides is observed, but no additive rules can be derived because of the structural diversity of the individual compounds studied.

As only a few Δn_{ci} and Δq_i contributions are known from previous studies[16], it was necessary to determine eleven values of contributions for phenylurea herbicides (fifteen compounds) and three Δn_{ci} and one Δq_i contributions for triazine herbicides, using the experimental data. All the n_{ce} and q_i indices calculated from the additive contributions agree well with the values determined directly from the experimental data and only two calculated n_{ce} values for phenylureas and two n_{ce} values for triazines differ from the experimental values by slightly more than 0.1 and the differences for all the calculated q_i indices from the experimental q_i values are less than 0.06. These results show that additive rules (eqns. 4a and b) can be used to predict the indices n_{ce} and q_i. Of course, only a limited number of compounds were tested, and it is possible that the additive rules may fail for some other compounds.

Prediction of retention in isocratic and gradient-elution chromatography

To test the practical usefulness of the n_{ce} and q_i indices for the prediction of retention in isocratic reversed-phase chromatography of phenylurea and triazine herbicides and related compounds, experimental retention volumes at four different compositions of methanol–water mobile phase were compared with V_R values calculated with the aid of eqn. 1 using the n_{ce} and q_i indices calculated by application of additive rules from eqns. 4a and b (Table III). The results are shown in Table IVA. About 75% of the calculated V_R values differ from the experimental values by less than 5% relative and 91% of the values differ by less than 10% relative.

In another set of experiments, some phenylurea herbicides were chromatographed on column B with a larger diameter and the experimental retention volumes under isocratic conditions were compared with V_R calculated from eqn. 1 using the indices n_{ce} and q_i and the constants a_0, a_1 and p determined earlier on column A. The differences between the calculated and measured retention volumes were less than 5% relative for approximately 80% of the values (Table IVB). An example of the isocratic separation of phenylurea herbicides is shown in Fig. 2.

It was the main purpose of this work to test possibilities for the prediction of

TABLE IV

EXPERIMENTAL RETENTION VOLUMES, $V_{R(e)}$, AND RETENTION VOLUMES CALCULATED USING EQN. 1 AND n_{ce} AND q_i INDICES CALCULATED BY APPLICATION OF ADDITIVE RULES (EQNS. 4a AND b), $V_{R(c)}$ IN METHANOL–WATER MOBILE PHASES UNDER ISOCRATIC CONDITIONS ON COLUMNS PACKED WITH SILASORB SPH C_{18} (7.5 μm)

c = % (v/v) of methanol in the mobile phase. Numbers of compounds as in Table I.

(A) Column 300 × 3.6 mm I.D., V_M = 2.42 cm³:

Type	Compound No.	c = 70		c = 60		c = 50		c = 40	
		$V_{R(e)}$	$V_{R(c)}$	$V_{R(e)}$	$V_{R(c)}$	$V_{R(e)}$	$V_{R(c)}$	$V_{R(e)}$	$V_{R(c)}$
Phenylurea herbicides	6	2.7	2.6	2.8	2.8	3.5	3.2	4.4	4.3
	7	3.0	2.9	3.5	3.4	4.4	4.3	5.8	6.0
	8	3.2	3.2	4.1	4.2	5.9	6.5	9.8	11.7
	9	4.3	4.3	7.1	6.8	13.5	12.9	27.2	27.2
	10	4.9	5.0	8.8	8.9	19.0	18.6	44.0	42.9
	11	5.9	6.1	12.4	12.4	30.5	29.7	–	76.8
	12	6.4	6.6	14.1	13.9	35.7	34.1	–	90.0
	13	9.3	9.7	26.6	25.9	–	78.4	–	248.4
	14	2.9	2.9	3.3	3.3	4.3	4.1	5.6	5.4
	15	4.0	4.0	6.7	6.4	13.6	12.7	28.6	28.7
	16	4.5	4.4	7.3	7.3	14.5	14.4	32.2	32.0
	17	4.2	4.1	6.5	6.4	12.3	11.8	25.7	24.6
	18	2.9	2.8	3.3	3.1	4.1	3.8	5.7	5.2
	19	4.5	4.3	7.6	6.9	14.3	13.2	32.3	28.5
	20	4.6	4.4	8.2	7.7	17.2	16.1	41.3	38.0
Triazine herbicides	28	3.9	3.8	5.5	5.4	9.1	9.0	17.6	16.7
	29	4.7	4.6	7.8	7.6	15.5	14.9	35.1	32.7
	30	6.1	6.1	13.0	12.6	33.1	30.4	–	79.3
	31	5.6	6.4	11.2	13.6	29.0	33.8	83.7	90.5
	32	4.8	4.7	8.2	7.6	17.3	14.4	38.7	30.3
	33	7.9	8.0	18.1	18.5	54.2	49.4	–	139.4
	34	8.4	8.6	20.7	20.8	61.9	57.3	–	166.3

(B) Column 300 × 4.1 mm I.D., V_M = 3.18 cm³:

Type	Compound No.	c = 60		Type	Compound No.	c = 54	
		$V_{R(e)}$	$V_{R(c)}$			$V_{R(e)}$	$V_{R(c)}$
Phenylurea herbicides	6	3.7	3.6	Triazine herbicides	28	9.1	9.5
	7	4.6	4.5		29	14.3	14.7
	8	5.3	5.5		30	27.0	27.7
	10	12.0	11.7		31	23.5	26.9
	11	16.4	16.3		32	15.9	14.2
	12	18.5	18.2		33	43.3	43.2
	13	37.4	34.0		34	50.7	49.7

Fig. 2. Isocratic separation of phenylurea herbicides on a Silasorb SPH C_{18} (7.5 μm) column (300 × 0.41 mm I.D.), V_M = 3.18 cm^3, using elution with 60% (v/v) methanol in water at 0.95 cm^3 min^{-1}. Detection: UV, 254 nm, 0.1 a.u.f.s. Compounds separated: 1 = hydroxymetoxuron; 2 = fenuron; 3 = metoxuron; 4 = impurity; 5 = diuron; 6 = linuron; 7 = chlorbromuron; 8 = neburon.

retention in gradient-elution chromatography. Eleven phenylurea herbicides were chromatographed on column B using several linear gradients with different profiles (initial concentration of methanol in mobile phase, A, and slope, B, i.e., the rate of change of methanol concentration with the volume of eluate). Figs. 3–5 show the chromatograms obtained in the experiments with three different gradient profiles. The gradient-elution instrument used caused a gradient delay volume of 2.25 ml as determined in an independent experiment with the U 6K injector connected directly to the detector (to avoid possible errors in mobile phase composition, the injector was left in the position "Inject" from the start to the end of the gradient run). It was assumed that the solutes do not move down the column during the delay period when the mobile phase of the initial composition flows through the column, as they are very strongly retained under these conditions. Consequently, it is sufficient to add the delay volume, V_d, to the calculated retention volumes for comparison with experimentally measured values[47]: $V_g = V_g'' + V_M + V_d$.

Table V shows the experimental V_g and two sets of retention volumes calculated from eqn. 10: (a) using experimental n_{ce} and q_i indices and (b) using n_{ce} and q_i indices calculated from additive contributions (eqns. 4a and b). The constants a_0, a_1 and p and the indices determined using column A were used in the calculations (Tables II and III). Both sets of calculated retention volumes predict accurately the
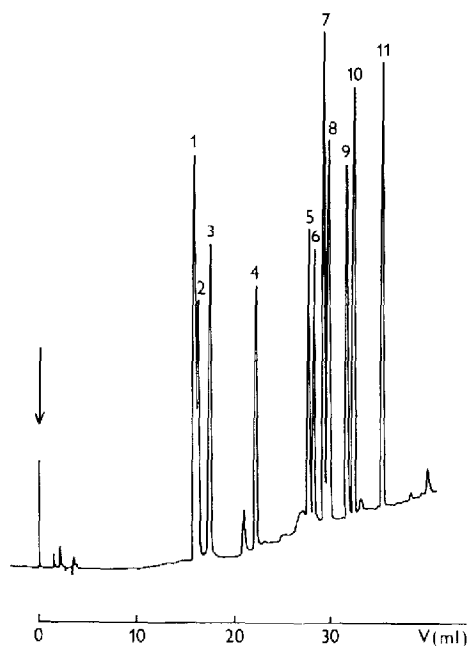
Fig. 3. Gradient-elution separation of phenylurea herbicides using a linear gradient from 15 to 100% methanol in 45 min at 0.95 cm³ min⁻¹; A = 0.15; B = 0.01997. Other conditions as in Fig. 2. Compounds separated: 1 = hydroxymetoxuron; 2 = desfenuron; 3 = fenuron; 4 = metoxuron; 5 = fluometuron; 6 = chlortoluron; 7 = isoproturon; 8 = diuron; 9 = linuron; 10 = chlorbromuron; 11 = neburon.
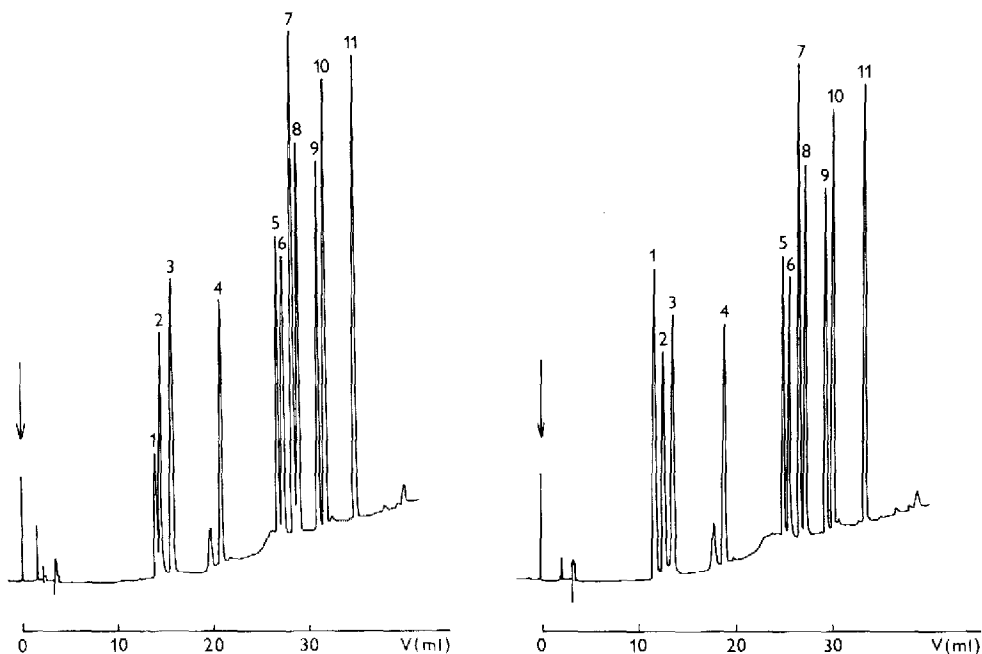

Fig. 4. Gradient-elution separation of phenylurea herbicides using a linear gradient from 20 to 100% methanol in 45 min at 0.97 cm³ min⁻¹; A = 0.2; B = 0.01835. Other conditions as in Fig. 2, compounds as in Fig. 3.

Fig. 5. Gradient-elution separation of phenylurea herbicides using a linear gradient from 25 to 100% methanol in 45 min at 0.95 cm³ min⁻¹; A = 0.25; B = 0.01749. Other conditions as in Fig. 2, compounds as in Fig. 3.

TABLE V

EXPERIMENTAL (exp.) AND CALCULATED (calc.) RETENTION VOLUMES, V_g, BAND WIDTHS, w_g, (BOTH IN cm³) AND RESOLUTION, $R_{i,j}$, OF PHENYLUREA HERBICIDES IN CHROMATOGRAPHY ON SILASORB SPH C$_{18}$ (7.5 μm) COLUMN (300 × 4.1 mm I.D., V_M = 3.18 cm³) USING ELUTION WITH VARIOUS LINEAR GRADIENTS OF METHANOL IN WATER

(I) 20–100% CH$_3$OH in 30 min, 0.98 cm³ min^{-1}; (II) 20–100% CH$_3$OH in 45 min, 0.97 cm³ min^{-1}; (III) 15–100% CH$_3$OH in 45 min, 0.95 cm³ min^{-1}; (IV) 25–100% CH$_3$OH in 45 min, 0.95 cm³ min^{-1}. A and B are the constants of gradient function (eqn. 7). Eqns. 10 and 8 were used for calculations. n_{ce} and q_i indices: (a) calculated using experimental a and m constants (eqns. 9a and b), (b) calculated using additive rules (eqns. 4a and b). The delay volume of the equipment V_d = 2.25 cm³ was added to the calculated values. w_g (exp) = 0.5 cm³.

Gradient	Compound No.	V_g Exp.	V_g Calc. (a)	V_g Calc. (b)	w_g calc.	$R_{i,j}$ Exp.	$R_{i,j}$ Calc. (a)	$R_{i,j}$ Calc. (b)
I	6	13.1	11.7	11.7	0.39			
(A = 0.2; B =	14	13.7	12.5	12.5	0.45	1.2	1.7	1.7
0.02718)	7	14.5	13.4	13.3	0.45	1.5	1.8	1.9
	8	18.2	17.0	17.6	0.45	7.5	8.0	9.5
	15	22.2	21.4	21.4	0.43	8.1	10.2	8.8
	16	22.7	21.9	22.1	0.44	1.0	1.1	1.6
	20	23.3	22.7	22.4	0.43	1.2	1.9	0.7
	10	23.7	23.1	23.2	0.43	0.9	0.9	1.8
	11	25.0	24.8	24.9	0.42	2.6	4.0	4.0
	12	25.5	25.3	25.3	0.41	1.0	1.2	1.0
	13	27.4	27.2	27.3	0.38	3.7	5.0	5.2
II	6	14.2	12.9	12.9	0.46			
(A = 0.2; B =	14	14.7	13.6	13.6	0.52	1.0	1.3	1.3
0.01835)	7	16.0	14.8	14.8	0.54	2.6	2.2	2.2
	8	21.2	20.2	21.0	0.55	10.4	9.8	11.3
	15	27.1	26.8	26.8	0.54	11.8	12.2	10.7
	16	27.7	27.6	27.7	0.55	1.2	1.4	1.6
	20	28.8	28.7	28.4	0.54	2.2	2.0	1.4
	10	29.3	29.3	29.5	0.55	1.0	1.1	2.0
	11	31.4	31.9	32.1	0.52	4.2	5.0	5.0
	12	32.2	32.7	32.8	0.51	1.6	1.6	1.4
	13	35.3	35.9	36.0	0.48	6.2	6.6	6.6
III	6	15.8	14.6	14.6	0.47			
(A = 0.15; B =	14	16.2	15.0	15.0	0.54	0.8	0.7	0.7
0.01997)	7	17.4	16.3	16.3	0.55	2.4	2.4	2.4
	8	22.1	21.7	22.6	0.54	9.4	10.0	11.6
	15	27.5	28.0	28.0	0.51	10.8	12.4	10.6
	16	28.1	28.7	28.8	0.52	1.2	1.3	1.5
	20	29.1	29.7	29.5	0.51	2.0	2.0	1.4
	10	29.6	30.3	30.4	0.52	1.0	1.1	1.7
	11	31.4	32.7	32.8	0.50	3.6	4.8	4.8
	12	32.1	33.4	33.5	0.49	1.4	1.4	1.4
	13	35.0	36.3	36.4	0.45	5.8	6.4	6.4
IV	6	11.7	11.2	11.3	0.43			
(A = 0.25; B =	14	12.7	12.2	12.2	0.49	2.0	1.9	1.8
0.01749)	7	13.6	13.2	13.2	0.51	1.8	2.0	1.9
	8	18.9	18.1	19.0	0.55	10.6	8.9	10.5
	15	24.9	24.9	24.9	0.55	12.0	12.3	10.7
	16	25.6	25.6	25.8	0.56	1.4	1.4	1.6
	20	26.6	26.8	26.5	0.55	2.0	2.2	1.3
	10	27.3	27.4	27.6	0.56	1.4	1.2	1.9
	11	29.4	30.1	30.3	0.54	4.2	5.0	5.0
	12	30.1	31.0	31.0	0.53	1.4	1.7	1.3
	13	33.4	34.3	34.4	0.49	6.6	6.7	6.9

order of elution and, with only one exception, the differences between the calculated and experimental V_g values are less than 10% relative. Band widths w_g calculated from eqns. 8, 9a and 9b, experimental and calculated values of the resolution, $R_{i,j}$, are also included in Table V (approximately constant experimental band widths of 0.5 cm^3 were considered in the experimental resolution):

$$R_{i,j} = \frac{V'_{g,j} - V'_{g,i}}{w_{g,j}} \tag{11}$$

The experimental and calculated resolution values are in acceptable agreement if the n_{ce} and q_i indices calculated from eqns. 9a and b and experimental a and m constants are used for calculation. Some $R_{i,j}$ values calculated using the indices n_{ce} and q_i determined by application of additive rules (eqns. 4a and b) may deviate significantly from the experimental values, such as the resolution for the pairs chlortoluron–isoproturon, isoproturon–diuron, fenuron–metoxuron and metoxuron–fluometuron.

It can be concluded that the calculations based on the indices n_{ce} and q_i determined from the additive contributions of structural elements allow one to predict accurately the order of elution and to calculate the retention volumes in gradient-elution HPLC with an acceptable error; however, the calculated resolution of adjacent peaks with small differences in V_g has too low a precision for calculations of most suitable gradient profiles for a given separation.

Selection of the gradient profile by adjusting the selectivity of separation using the slope of the gradient and the initial composition of the mobile phase

As shown in the Theoretical section, the selectivity of separation in gradient-elution chromatography may be characterized using the differences in retention volumes, ΔV_g, rather than using the ratios of retention volumes. ΔV_g depends on the Δn_c, Δq, $n_{ce,i}$ and $q_{i,i}$ indices of the solutes and also on the gradient profile, which is defined by the initial composition of the mobile phase and the slope of the linear gradient.

It is possible to control the isocratic selectivity of separation of a given pair of compounds with different slopes of log k' *versus* x plots to a certain extent by adjusting the concentration of the organic solvent in a binary aqueous–organic mobile phase, x[17]. A disadvantage of this approach is substantial change in absolute retention when x changes in binary mobile phases. This can be avoided in gradient-elution chromatography by adjusting simultaneously the initial mobile phase composition (A) and the slope of the linear gradient (B), which may result in a change in separation selectivity for certain pairs of solutes with only minor changes in absolute retention if the time of the gradient is constant. Although ternary or more complex multisolvent gradients are more generally applicable for controlling the selectivity of separation[48–51], simultaneous adjustment of A and B may be very useful for selectivity tuning of certain "tailor-made" gradient elution separations, as it requires only a binary solvent gradient.

If the optimal gradient profile is desired at a constant gradient time, t_G (or at a constant gradient volume, $V_G = t_G F_m$, where F_m is the flow-rate of the mobile phase), a change in one of the parameters A or B necessitates a simultaneous ad-

justment of the other parameter, as can be seen from re-arranged eqn. 7:

$$B = \frac{x_G - A}{V_G} \tag{12}$$

where x_G is the concentration of the organic solvent, x, in the mobile phase at the end of the gradient elution, where the volume of eluate is equal to V_G.

This approach to optimization of a binary gradient profile is illustrated by the example of the reversed-phase gradient-elution separation of eleven phenylurea herbicides. Using n_{ce} and q_i indices determined experimentally on column A, gradient-elution volumes and resolution, $R_{i,j}$, on column B were predicted by calculation from eqns. 8–11 for various combinations of the gradient parameters A and B, related to one another by eqn. 12. V_G was pre-set to 45 ml, which corresponds to the 45 min gradient at $F_m = 1$ cm^3 min^{-1}. The resolution map for the pairs of compounds with adjacent peaks is shown in Fig. 6 for various initial concentrations of methanol in
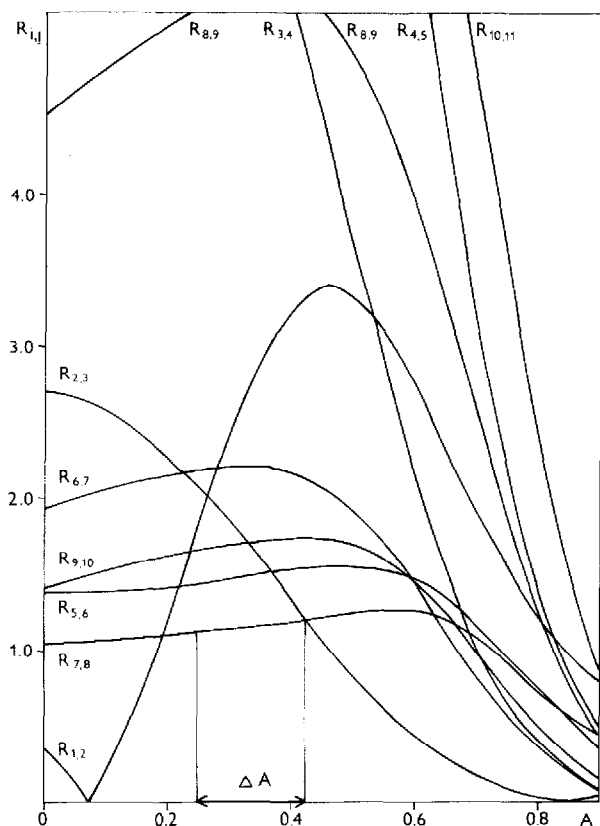
Fig. 6. Predicted resolution map for adjacent pairs of compounds (phenylurea herbicides) in gradient-elution chromatography with linear concentration gradients of methanol in water. Column as in Fig. 2. The initial concentration of methanol in the mobile phase, A, is related to the gradient slope by eqn. 12 (constant gradient volume $V_G = 45$ ml is pre-set). $R_{i,j}$, the resolution of the compounds with adjacent peaks, i and j, is calculated from eqns. 8–11. Compounds as in Fig. 3. ΔA is the interval of initial concentrations expected to yield the best resolution of the eleven compounds in the mixture.

the mobile phase, A. A reversal of the order of elution is predicted at $A < 0.07$ for compounds 1 and 2 and at $A > 0.85$ for compounds 2 and 3. The separation of solutes 1 and 2 is predicted to be insufficient at $A < 0.2$ and that of solutes 2 and 3 at $A > 0.42$. In the region of A from 0.2 to 0.42, the resolution of solutes 7 and 8 is predicted to be critical and it slowly increases with increasing A. The optimal resolution of the mixture of eleven phenylurea herbicides is to be expected at $A = 0.42$, *i.e.*, for linear solvent gradients from 42 to 100% methanol in water in 45 min. If $R_{i,j} \geqslant 1.15$ is acceptable, the initial concentration of methanol can be selected between 25 and 42%.

Three chromatograms obtained at different initial mobile phase compositions ($A = 0.15$, 0.2 and 0.25) shown in Figs. 3–5 are in agreement with the predicted resolution map. The retention time of the last compound eluted is approximately constant and independent of A. Increasing A at a constant V_G improves the resolution of compounds 1 and 2 (hydroxymetoxuron and desfenuron) and of compounds 7 and 8 (isoproturon and diuron). An experimental gradient starting at 25% methanol yielded an acceptable separation of all the eleven phenylureas (Fig. 5).

CONCLUSIONS

The indices n_{ce} and q_i of phenylurea and triazine herbicides can be predicted using additive rules. The additive contributions to n_{ce} and q_i of the individual structural elements (functional groups) are in rough agreement with the increments determined earlier for simple substituted benzenes.

The indices n_{ce} and q_i of the herbicides studied, calculated by application of the additive rules, can be used to predict retention volumes, V_R, in both isocratic and gradient-elution chromatography, by calculation. The order of elution is predicted correctly and the error in the calculated V_R values is less than 10% relative for most compounds. This calculation approach can be applied with good results to columns with dimensions differing from those of the columns used for the determination of n_{ce} and q_i. The resolution can be also calculated with reasonable precision using this approach; however, the errors in the resolution calculated from n_{ce} and q_i indices determined by application of the additive rules may be too large for fine tuning of the separation of certain solutes by calculation of the optimal conditions.

Correct simultaneous adjustment of the initial mobile phase composition and the slope of linear concentration gradients in reversed-phase chromatography is an efficient tool for optimization of separation selectivity for compounds with different slopes of log k' *versus* x plots, which may be used successfully as a simple, but less general, alternative to multi-solvent gradient optimization.

REFERENCES

1 Cs. Horváth, W. Melander and I. Molnár, *J. Chromatogr.*, 125 (1976) 129.
2 Cs. Horváth, W. Melander and I. Molnár, *Anal. Chem.*, 49 (1977) 142.
3 D. E. Martire and R. E. Boehm, *J. Liq. Chromatogr.*, 3 (1980) 753.
4 K. Jinno and K. Kawasaki, *J. Chromatogr.*, 316 (1984) 1.
5 K. Jinno and K. Kawasaki, *J. Chromatogr.*, 298 (1984) 326.
6 K. Jinno and K. Kawasaki, *Chromatographia*, 17 (1983) 337, 445.
7 K. Jinno and K. Kawasaki, *Chromatographia*, 18 (1984) 90, 211.

8 T. L. Hafkenscheid and E. Tomlinson, *J. Chromatogr.*, 264 (1983) 47.
9 R. M. Smith, *J. Chromatogr.*, 236 (1982) 313.
10 R. M. Smith, *Anal. Chem.*, 56 (1984) 256.
11 J. K. Baker, *Anal. Chem.*, 51 (1979) 1693.
12 J. K. Baker and C.-Y. Ma, *J. Chromatogr.*, 169 (1979) 107.
13 P. Jandera, H. Colin and G. Guiochon, *Anal. Chem.*, 54 (1982) 435.
14 H. Colin, G. Guiochon and P. Jandera, *Anal. Chem.*, 55 (1983) 442.
15 P. Jandera, *Chromatographia*, 19 (1984) 101.
16 P. Jandera, *J. Chromatogr.*, 352 (1986) 91.
17 P. Jandera, *J. Chromatogr.*, 352 (1986) 111.
18 J. Pribyl, *Chromatographia*, 10 (1977) 753.
19 G. Glad, T. Popoff and O. Theander, *J. Chromatogr. Sci.*, 16 (1978) 118.
20 J. Pribyl and F. Herzel, *J. Chromatogr.*, 166 (1978) 272.
21 U. A. Th. Brinkman, A. De Kok and R. B. Geerdink, *J. Chromatogr.*, 283 (1983) 113.
22 T. H. Byast, *J. Chromatogr.*, 134 (1977) 216.
23 J. H. Kennedy, *J. Chromatogr. Sci.*, 15 (1977) 79.
24 J. F. Lawrence, *J. Chromatogr.*, 211 (1981) 144.
25 E. G. Cotterill, *J. Chromatogr.*, 197 (1980) 267.
26 B. Rittich and H. Dubský, *J. Chromatogr.*, 209 (1981) 7.
27 G. Chiavari and C. Bergamini, *J. Chromatogr.*, 346 (1985) 369.
28 T. Braumann, G. Weber and L. H. Grimme, *J. Chromatogr.*, 261 (1983) 329.
29 J. D. Mattice and T. L. Lavy, *J. Chromatogr.*, 250 (1982) 109.
30 C. E. Goewie, P. Kwakman, R. W. Frei, U. A. Th. Brinkman, W. Maasfeld, T. Seshadri and A. Kettrup, *J. Chromatogr.*, 284 (1984) 73.
31 G. Kulshrestha and R. Khazanchi, *J. Chromatogr.*, 318 (1985) 144.
32 S. M. Walters, B. C. Westerby and D. M. Gilvydis, *J. Chromatogr.*, 317 (1985) 533.
33 M. W. F. Nielen, G. Koomen, R. W. Frei and U. A. Th. Brinkman, *J. Liq. Chromatogr.*, 8 (1985) 315.
34 M. Furukawa and T. Tokoyama, *J. Chromatogr.*, 208 (1981) 424.
35 P. Beilstein, A. M. Cook and R. Hutter, *J. Agric. Food Chem.*, 29 (1981) 1132.
36 C. E. Parker, C. A. Hanley, D. J. Harvan and J. R. Hass, *J. Chromatogr.*, 242 (1982) 77.
37 C. E. Parker, G. H. Degen, E. D. Abusteit and F. T. Corbin, *J. Liq. Chromatogr.*, 6 (1983) 725.
38 D. J. Subach, *Chromatographia*, 14 (1981) 371.
39 N. M. J. Vermeulen, Z. Apostolides, D. J. J. Potgieter, P. C. Nel and N. S. H. Smit, *J. Chromatogr.*, 240 (1982) 247.
40 K. A. Ramsteiner and W. D. Hoermann, *J. Agric. Food Chem.*, 27 (1979) 934.
41 P. Dufek and V. Pacáková, *J. Chromatogr.*, 187 (1980) 341.
42 E. Smolková and V. Pacáková, *Chromatographia*, 11 (1978) 698.
43 P. Dufek, V. Pacáková and E. Tesařová, *J. Chromatogr.*, 191 (1980) 115.
44 P. Jandera, *J. Chromatogr.*, 314 (1984) 13.
45 P. Jandera and J. Churáček, *J. Chromatogr.*, 91 (1974) 223.
46 P. Jandera, J. Churáček and L. Svoboda, *J. Chromatogr.*, 192 (1980) 37.
47 P. Jandera and J. Churáček, *Gradient Elution in Column Liquid Chromatography*, Elsevier, Amsterdam, 1985.
48 L. R. Snyder, in Cs. Horváth (Editor), *High-Performance Liquid Chromatography, Advances and Perspectives*, Vol. 1, Academic Press, New York, 1980, p. 207.
49 P. Jandera, J. Churáček and H. Colin, *J. Chromatogr.*, 214 (1981) 35.
50 J. J. Kirkland and J. L. Glajch, *J. Chromatogr.*, 255 (1983) 27.
51 J. L. Glajch and J. J. Kirkland, *Anal. Chem.*, 54 (1983) 2593.